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Increasing Fish Oil Levels in Commercial Diets Influences Hematological and Immunological Responses of Channel Catfish, *Ictalurus punctatus*

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Abstract

Growth performance, immune responses and disease resistance were studied in juvenile channel catfish, *Ictalurus punctatus*, fed a commercial diet (35.3% crude protein and 5.6% lipid) supplemented with menhaden fish oil at levels of 0, 3, 6, and 9% for 15 wk. Dietary fish oil levels did not significantly influence growth performance of catfish. Fatty acid compositions of whole-body and liver reflected dietary fatty acid composition. No differences were found in hematological values, except that fish fed the 9% fish oil diet had significantly lower hematocrit. The resistance of erythrocytes to hemolysis in hypotonic solutions increased with increasing fish oil levels and the highest resistance was seen with the 9% fish oil diet. Fish fed 6 and 9% added fish oil diets had significantly higher serum protein levels than that of control fish. Serum lysozyme activity of fish fed 3 and 6% added fish oil diet was significantly higher than that of the control. Complement activity and chemotaxis ratio significantly decreased in fish fed diets with 6 or 9% added fish oil. The 3% added fish oil diet, however, had significantly highest natural hemolytic complement activity. Mortality from *Edwardsiella ictaluri* 14 d postchallenge and antibody titers to *E. ictaluri* did not differ among treatments.

In general, freshwater fish require dietary sources of polyunsaturated fatty acids of the n-6 (linoleic acid, 18:2 n-6) and n-3 (linolenic acid, 18:2 n-3) families for optimum growth. Based on their essential fatty acid requirements, cultured freshwater fish, including channel catfish, are commonly fed grain–soybean meal feeds high in n-6 fatty acids. Based on their essential fatty acid requirement, channel catfish should be fed diets containing 0.5–1.0% n-3 fatty acids (NRC 1993). Feeding studies have shown that channel catfish fed diets supplemented with fish oil had body fatty acid composition, and the ratio of n-3 to n-6 fatty acids positively correlated to their levels in the diets (Abdel-Aty Mohamed 1989; Li et al. 1994; Fracalossi and Lovell 1995; Manning and Li 2002).

Suboptimal and/or excessive levels of certain nutrients, including n-3 and n-6 fatty acids, may adversely affect immune response and disease resistance of fish (Lim and Webster 2001). Optimum functioning of the immune system depends on balanced and adequate nutrition. N-3 highly unsaturated fatty acids (HUFAs) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are readily incorporated into polar lipids in cellular membranes. Their presence in the cell membrane affects the functioning of immune cells. Increased intracellular killing of the bacteria *Edwardsiella ictaluri*, the cause of enteric septicemia of catfish, by head kidney macrophages has been demonstrated in channel catfish fed diets containing high levels of n-3 HUFAs (Sheldon and Blazer 1991). Fracalossi and Lovell (1994) obtained increased antibody titers 2 wk after immunization against *E. ictaluri* in catfish fed a fish oil diet compared with those fed diets containing corn oil, linseed oil, or a mixture of fish oil, corn oil and beef tallow. High dietary levels of n-3 HUFAs, however, can negatively affect fish growth as these fatty acids are readily oxidized by reactive oxygen species to lipid peroxides (Porter et al. 1995). Red seabream juveniles fed high levels of n-3 HUFAs showed low growth rates (Takeuchi et al. 1992). Fracalossi and Lovell (1994) and Li et al. (1994) reported that dietary

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menhaden oil (rich in n-3 HUFA) and linseed oil (rich in 18:3n-3) increased the susceptibility of channel catfish to *E. ictaluri*. Erdal et al. (1991) showed that increased levels of dietary n-3 HUFAs also reduced survival of Atlantic salmon when the fish where subjected to *Yersinia ruckeri* challenge.

Therefore, the objectives of this study were to evaluate the effects of various levels of menhaden fish oil supplemented to a commercial diet on growth performance, hematology, immune response, and resistance of channel catfish to *E. ictaluri* challenge.

Materials and Methods

Experimental Fish and Rearing Facilities

National Warmwater Aquaculture Center 103 strain channel catfish, *Ictalurus punctatus*, fingerlings were acclimated to a commercial floating catfish feed for 2 wk before stocking. At the end of the acclimation period, fish (average weight of 14.56 ± 0.23 g) were randomly stocked into sixteen 110-L aquaria at a density of 50 fish per aquarium. Aquaria were supplied with flow-through dechlorinated, heated municipal city water at an initial rate of about 0.6 L/min and increased gradually to about 1.0 L/min by the end of the study. Water was continuously aerated using air stones. Water temperature and dissolved oxygen in four randomly chosen aquaria were measured once every other day in the morning, using a YSI model 58 Oxygen Meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). During the trial, water temperature averaged 26 ± 0.04 C and dissolved oxygen averaged 4.9 ± 0.04 mg/L. Photoperiod was maintained at a 12:12 h light : dark schedule.

Feeding and Sampling

A commercial floating catfish feed containing 35.3% crude protein and 5.6% crude lipid (CO-OP, Products, Alabama Farmers Cooperative, Inc., Decatur, AL, USA) was sprayed with menhaden fish oil (ARBP refined menhaden oil; Omega Protein, Inc., Reedville, VA, USA) at levels of 0, 3, 6, and 9% of diets and thoroughly mixed in a Hobart mixer (Hobart Corporation, Troy, OH, USA). Diets were stored in plastic bags at −20 C until needed. The proximate and fatty acid composition of the experimental diets are presented in Table 1.

Fish in four randomly assigned aquaria were fed one of the four experimental diets twice daily (between 0730–0830 and 1500–1600 h) to apparent satiation for 15 wk. The amount of diet consumed was recorded daily by calculating the differences in weight of diets before the first and after the last feeding. Once a week, aquaria were scrubbed and accumulated waste was siphoned. On cleaning days, fish were fed only in the afternoon. Fish in each aquarium were group weighed and counted at 3-week intervals. Feed was not offered on sampling days.

Fatty Acid Analysis

Lipids from the experimental diets, whole-body and liver samples were extracted following the methanol/chloroform modified method of Folch et al. (1957). Pooled samples of four fish from each tank were ground in a Hobart meat grinder (Hobart Corporation, Troy, OH, USA). A 1-g subsample from each tissue (ground whole fish or liver) and diets was used for lipid extraction. After a series of liquid–liquid phase separations, centrifugation, and evaporation under nitrogen, the lipid fractions were methylated with boron trifluoride–methanol solution. Fatty acid methyl esters were analyzed with a Perkin-Elmer Clarus-500 gas chromatograph with a mass spectrometry detector (Perkin-Elmer, Shelton, CT, USA) as described in Yildirim-Aksoy et al. (2007a). Relative concentration of fatty acids was calculated and expressed as mass percentages of the identified fatty acids.

Hematological Assay and Erythrocyte Osmotic Fragility

At the end of the feeding period, four fish were randomly chosen from each tank and anesthetized with tricaine methanesulfate (MS-222) at 150 mg/L. Blood samples were collected from caudal vasculature with dried heparinized (100 IU) tuberculin syringes for hematological assays. Red and white blood cell counts were performed in duplicate for each sample by diluting whole blood (1:10,000) and enumerating in

TABLE 1. *Fatty acid composition (g/kg of diet) and proximate composition of experimental diets.*[1]

	Experimental diets			
	Control	C+3%	C+6%	C+9%
14:0	1.07	2.79	5.19	7.62
15:0	0.11	0.13	0.21	0.53
16:0	11.19	18.06	23.51	27.49
18:0	3.17	4.13	5.14	5.96
16:1 n-7	1.82	4.71	8.37	11.13
18:1 n-7	1.01	1.88	2.91	3.64
18:1 n-9	13.83	15.68	18.43	22.06
20:1 n-9	0.28	0.29	0.74	1.26
18:2 n-6	18.96	23.14	23.09	21.40
20:2 n-6	0.06	0.08	0.16	0.27
18:3 n-6	0.03	0.04	0.21	0.27
20:3 n-6	0.06	0.04	0.11	0.13
20:4 n-6	0.17	0.17	0.48	0.66
22:5 n-6	0.06	0.13	0.21	0.07
18:3n-3	1.49	2.13	2.49	2.65
20:3 n-3	0.00	0.04	0.11	0.07
18.4 n-3	0.17	0.92	1.85	2.65
20:4 n-3	0.11	0.58	1.01	1.46
20:5 n-3	1.23	3.67	3.34	9.87
22:5 n-3	0.31	0.50	0.69	2.12
22:6 n-3	0.93	4.17	7.73	11.13
Σ saturates	15.54	25.10	34.05	41.61
Σ monoenes	16.94	22.56	30.45	38.09
Σ n-6	19.33	23.60	24.25	22.79
Σ n-3	4.24	12.01	17.21	29.95
Σ n-3 HUFA[2]	2.58	8.97	12.87	24.65
n-3/n-6	0.12	0.43	0.75	1.74
Proximate composition (% air dry weight)				
Dry matter	93.39	93.73	94.25	94.72
Protein	35.31	34.8	33.89	33.20
Lipid	5.61	8.34	10.59	13.25
Ash	6.92	6.69	6.56	6.27

HUFA = highly unsaturated fatty acids.

[1] Values reported are means of two determinations per diet.

[2] n-3 fatty acids with 20 carbons or more.

a Spencer Bright Line hemacytometer. Hematocrit of each fish was determined in duplicate using the microhematocrit method (Brown 1988). Hemoglobin was determined using a cyanomethemoglobin method (Point Scientific, St. Louis, MO, USA). Hemoglobin values were adjusted by cyanomethemoglobin correction factor for channel catfish described by Larsen (1964).

Erythrocyte osmotic fragility from each of the four fish per tank was performed using the method of gradient saline solutions. Briefly 6 μL of blood was added to 2 mL salt (NaCl) solutions (0, 0.25, 0.45, 0.55, 0.65, and 0.85%) using 24-well plates. After incubation at room temperature for 30 min, the plates were centrifuged at 800 *g* for 10 min. Supernatant was transferred to 96-well microtiter plates and the absorbance of the samples was read at 415 nm. The percent hemolysis was calculated relative to the 100% hemolysis values obtained from the 0% saline.

Nonspecific Immune Responses

Serum total protein level and lysozyme activity were performed as described in Yildirim-Aksoy et al. (2007b). Serum from each of the four fish per tank was assayed in duplicate for serum total protein concentration using the

modified Biuret method. Serum total immunoglobulin was determined following the method of Siwicki and Anderson (1993). The assay was based on the measurement of total protein content in serum before and after precipitating the immunoglobulin molecules employing a 12% solution of polyethylene glycol. The difference in protein content is considered the total immunoglobulin content. Serum lysozyme activity was determined by the method of Litwack (1955) as modified by Sankaran and Gurnani (1972) by measuring the lytic activity of the catfish serum against bacterium *Micrococcus lysodeikticus* (Sigma Chemical Co., St. Louis, MO, USA).

Serum natural hemolytic (alternative pathway) complement activity was adapted from Sunyer and Tort (1995) and modified for use in microtiter plates as described in Yildirim-Aksoy et al. (2007b), except 0.85% phosphate-buffered saline (PBS) containing $MgCl_2$, $CaCl_2$ and gelatin (PBS^{3+}) was used instead of GVB^{2+}as the assay solution. This assay is based on the hemolysis of sheep erythrocyte (Remel Inc., Lenexa, KS, USA) by complement present in fish serum. Briefly, sheep erythrocytes were washed and standardized to 5×10^7 cells/mL in PBS^{3+} before use. Sheep erythrocyte cell suspension was added to serially diluted (1:2) serum in PBS^{3+} in a round-bottom 96-well microtiter plate. Positive (100% lysis) and negative controls (spontaneous lysis) were also processed in each plate by replacing serum with distilled water and buffers, respectively. Samples were incubated at room temperature (22 C) for 1 h with regular shaking. The plates were centrifuged at 800 *g* for 10 min at 4 C to avoid unlysed cells. Supernatants were transferred to a flat-bottom microtiter plate and the absorbance measured at 415 nm using an enzyme-linked immunosorbent assay plate reader. Complement hemolytic activity is expressed as ACH_{50} value, which represents the volume of serum necessary to produce lysis of 50% of the target cells under standard conditions, and the results are presented as units/mL.

Macrophage chemotaxis was performed after isolation and collection of peritoneal leucocytes (mostly macrophages) by a modification of the lower surface method (Boyden 1962) as described by Yildirim et al. (2003). The assay is based on the migration ratio of macrophages toward *E. ictaluri* exoantigen. Briefly, squaline-elicited peritoneal macrophages were collected from each of the five fish per tank using ice-cold 0.85% phosphate-buffered saline solution, pooled, centrifuged at 2000 *g*, and the cells were resuspended in calcium, magnesium, and sodium-free Hank's balanced salt solution (HBSS) without phenol red. Each sample was run in triplicate, with or without (control) *E. ictaluri* exoantigen, using chemotaxis chambers and 8-μm pore filters. Migrated macrophages were counted in five fields of triplicate filters at 100× after 90-min incubation at room temperature.

Bacterial Challenge and Antibody Production

Edwardsiella ictaluri (AL-93-75) from a frozen stock was grown in brain heart infusion (BHI) broth at 26 C for 24 h. The concentration of the culture was adjusted to an optical density of 1.74 at 540 nm to give an *E. ictaluri* concentration of approximately 2×10^{10} colony forming units/mL. To determine the optimum bacterial cell concentration to use in the experimental challenge, groups of 24 fish that were held in separate aquaria and fed the control diet for 12 wk, were challenged with 0, 1×10^7, 4×10^7, 8×10^7, and 1.6×10^8 *E. ictaluri* cells/mL. Water flow and feeding were discontinued for the first 6 and 24 h after challenge, respectively. Mortality was recorded twice daily for 15 d. The LC_{50} (concentration lethal to 50% of exposed fish) calculated by Karber's method (Plumb and Browser 1983) was 2×10^7 cells/mL and the concentration used for the experimental challenge. At the end of the 15-week feeding period, 20 fish from each aquarium were randomly selected and challenged with 2×10^7 cells/mL of *E. ictaluri* by immersion as described above. Each group of fish continued to receive their respective diets. Mortality was recorded twice daily for 15 d.

At the 15th day postchallenge, an additional four surviving fish per tank were bled using non-heparinized tuberculin syringes and allowed to clot at 4 C overnight. Serum samples were

collected following centrifugation and stored at −80 C until used. Agglutinating antibody titers against *E. ictaluri* in pre- and postchallenge serum samples were determined by modifying the method of Chen and Light (1994) as described in Yildirim et al. (2003). Data on prechallenged antibody titers indicated that all fish were negative for *E. ictaluri*. The agglutination end point was established as the last serum dilution in which cell agglutination was visible after incubation. Agglutination titers were reported as $\log_{10}$ of the reciprocal of the highest serum dilution showing visible agglutination compared with the positive control.

Statistical Analysis

Data were analyzed by ANOVA using the general linear model. If there was a significant *F*-test, subsequent comparisons of treatment means were determined using the Duncan's multiple range test. Differences were considered significant at the 0.05 probability level. All analysis was performed using the SAS program (2001; Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, USA).

Results

Final weight gain, feed intake, feed efficiency ratio and survival of channel catfish fed commercial diets were not significantly different among dietary treatments. Average final weight gains were 103.52, 101.01, 95.45, and 111.27 g for 0, 3, 6, and 9% added fish oil diets, respectively. Dry matter feed intake, feed efficiency ratio, and survival ranged from 118.4 to 126.0 g/fish, 0.81–0.88, and 95–100%, respectively.

The summaries of fatty acid profiles of whole-body and liver lipids are given in Figures 1 and 2, respectively. Fatty acid composition of both whole fish and liver lipids generally reflected the dietary fatty acid profile. Saturated fatty acids in whole fish were significantly increased, whereas monoenoic fatty acids were decreased in fish fed increasing levels of dietary fish oil. The values of these fatty acids in liver did not significantly differ. Total n-3 fatty acids and n-3 HUFAs in whole-body lipid significantly increased, whereas total n-6 fatty acids decreased at each incremental level of fish oil. The same trend was observed in liver

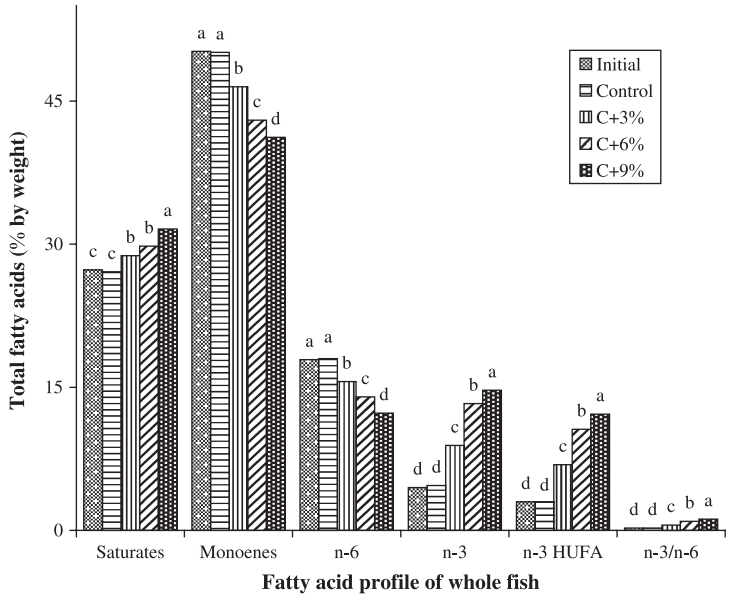

FIGURE 1. *Summary of fatty acid profiles of whole-body lipid of channel catfish fed commercial diets supplemented with various levels of fish oil for 15 wk. Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in same fatty acid classes with different superscripts are significantly different at* $P < 0.05$.

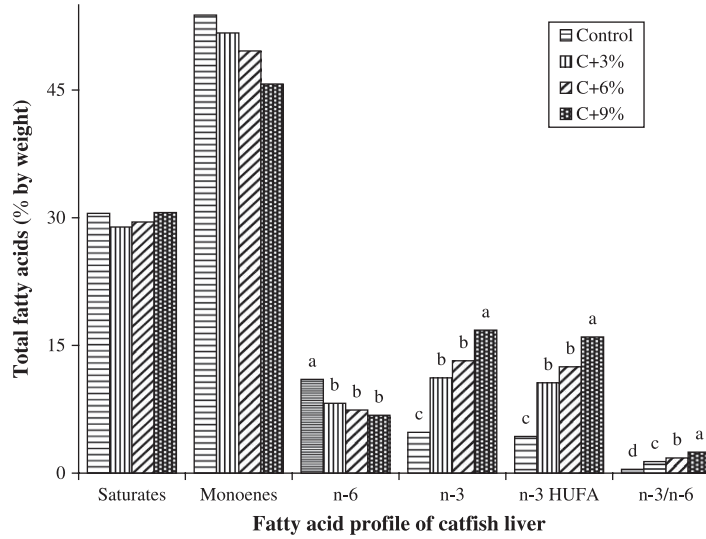

FIGURE 2. *Summary of fatty acid profiles of liver lipid of channel catfish fed commercial diets supplemented with various levels of fish oil for 15 wk. Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in same fatty acid classes with different superscripts are significantly different at* $P < 0.05$.

lipids, but the values were not always significantly different among treatments. As a result, the ratios of n-3/n-6 in both tissues significantly increased with increasing levels of dietary fish oil. Hematological parameters (red and white blood cell counts, and hemoglobin) were not affected by dietary fish oil levels, except hematocrit that was significantly lower in fish fed the 9% added fish oil diet compared with the groups fed the control or 3% added fish oil diets (Table 2).

Osmotic fragility of erythrocytes from fish fed experimental diets was distinctly different (Fig. 3). Erythrocytes from fish fed the diet with 9% added fish oil were the most resistant to hemolysis under hypotonic saline conditions. Susceptibility of erythrocytes to hemolysis progressively decreased with increasing fish oil supplementation and erythrocytes from fish fed the control diet were the most fragile. Salt concentrations in saline solutions that caused 50% erythrocyte hemolysis were significantly highest and lowest in fish fed the control and 9% fish oil diets, respectively (Table 2). The values for fish fed 3 and 6% added fish oil diets did not differ but were significantly higher and lower than those fed the control and 9% fish oil diets, respectively.

TABLE 2. *Hematological values (red blood cell count [RBC], white blood cell count [WBC], hemoglobin and hematocrit) of channel catfish fed commercial diets supplemented with various levels of fish oil for 15 wk.*[1]

Levels of added fish oil (%)	RBC[2] $\times 10^6/\mu L$	WBC[2] $\times 10^5/\mu L$	Hemoglobin[3] (g/dL)	Hematocrit[2] (%)	Salt concentration[4] SC_{50} (%)
0	2.49	3.46	8.57	36.93[a]	0.63[a]
3	2.48	3.75	8.70	36.23[a]	0.57[b]
6	2.43	3.64	8.10	34.48[ab]	0.55[b]
9	2.33	3.67	7.99	32.35[b]	0.50[c]
Pooled SEM	0.08	0.25	0.24	1.05	0.02

[1] Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2] Values are means of two determinations per fish, four fish per tank and four tanks per treatment.

[3] Values are means of one determination per fish, four fish per tank and four tanks per treatment.

[4] Salt concentrations in saline solutions causing 50% erythrocyte hemolysis. Values are means of one determination per fish, four fish per tank, and four tanks per treatment.

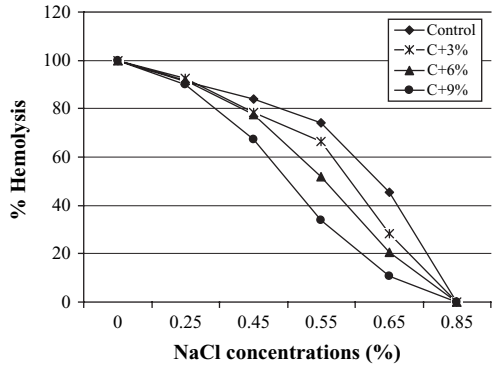

FIGURE 3. *Erythrocyte osmotic fragility of channel catfish fed commercial diets supplemented with various levels of fish oil for 15 wk. Values are means of one determination per fish, four fish per tank and four tanks per treatment.*

Serum protein, total immunoglobulin, lysozyme activity, natural hemolytic complement activity, and macrophage chemotaxis ratio are shown in Table 3. Serum protein concentration significantly increased in fish fed diet with added fish oil of 6% or higher. The value of this parameter was intermediate for fish fed the diet with 3% added fish oil. Total circulating immunoglobulin level was not significantly influenced by dietary levels of fish oil. Serum lysozyme activity of fish fed diets with 3 or 6% added fish oil was significantly higher than that of fish fed the control diet. Increasing the level of fish oil to 9% did not significantly stimulate lysozyme activity over that of the control. Natural hemolytic complement activity was significantly highest in fish fed the diet with 3% added fish oil. Increasing dietary fish oil levels to 6% or higher, however, significantly reduced complement activity compared with those of fish fed the control or 3% fish oil diets. There was a trend of decreasing macrophage chemotaxis ratio with increasing dietary fish oil level, and fish fed diets supplemented with 6 or 9% fish oil had significantly lower macrophage chemotaxis ratios than that of the control diet group.

Mean number of days at which the first mortality occurred after *E. ictaluri* challenge and agglutination antibody titer were not affected by dietary levels of added fish oil (Table 4). Cumulative mortality of fish fed the 6% added fish oil diet was significantly lower than those fed the 3 or 9% added fish oil diet, but these values did not differ from that of the control treatment.

Discussion

Although some earlier studies have shown increasing dietary HUFA levels improve growth and feed utilization efficiency (Santha and Gatlin 1991; Gatlin and Bai 1993), several other studies (Gatlin and Stickney 1982; Twibell and Wilson 2003; Lim et al. 2006) reported no growth enhancement in channel catfish fed high dietary levels of 18:3n-3 or n-3 HUFAs. In the present study, channel catfish fed the

TABLE 3. *Serum protein, total circulating immunoglobulin, lysozyme activity, natural hemolytic complement activity, and macrophage chemotaxis ratio of channel catfish fed commercial diets supplemented with various level of fish oil for 15 wk.*[1]

Levels of added fish oil (%)	Serum protein (mg/mL)[2]	Total immunoglobulin (mg/mL)[2]	Lysozyme activity (μg/mL)[2]	Alternative complement (units/mL)[3]	Macrophage chemotaxis ratio[4]
0	29.67[b]	5.78	2.93[b]	129.34[b]	0.86[a]
3	30.77[ab]	4.98	4.00[a]	136.94[a]	0.83[ab]
6	32.83[a]	5.39	4.19[a]	81.79[c]	0.81[b]
9	32.29[a]	6.09	3.31[ab]	54.57[d]	0.80[b]
Pooled SEM	0.75	0.60	0.29	3.26	0.01

[1] Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2] Values are means of two determinations per fish, four fish per tank, and four tanks per treatment.

[3] Dilution causing 50% hemolysis. Values are means of one determination per fish, four fish per tank, and four tanks per treatment.

[4] Values are means of three determinations per pooled sample of five fish per tank and four tanks per treatment. Chemotaxis ratio represents the number of migrating cells in the presence of *E. ictaluri* exoantigen divided by the sum of the number migrating cells without (control) and with *E. ictaluri* exoantigen.

TABLE 4. *Mean number of days to first mortality and cumulative mortality of channel catfish 15 d post-*E. ictaluri *challenge and antibody production against the same bacterium.*[1]

Level of added fish oil (%)	Days to first mortality	Cumulative mortality (%)	Antibody titer ($\log_{10}$)
0	6.75	58.8[ab]	2.01
3	6.25	76.3[a]	1.88
6	6.75	51.3[b]	1.79
9	6.25	76.3[a]	1.88
Pooled SEM	0.25	6.88	0.11

[1] Values are means of four replicates per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

commercial catfish diet (5.6% lipid) supplemented with fish oil at levels of 0, 3, 6, and 9% had similar growth, feed consumption, feed efficiency, and survival. Changes in fatty acid composition, specifically increase in n-3 HUFAs, were observed in both whole-body and liver lipids in response to increased dietary fish oil supplementation. Alteration of fatty acid composition of farmed fish lipids through diets has been reported in numerous studies (Abdel-Aty Mohamed 1989; Li et al. 1994; Fracalossi and Lovell 1995; Manning and Li 2002). Both linoleic and linolenic acids in whole-body and liver lipids reflected dietary levels of these fatty acids in this study. However, liver levels of n-3 HUFAs were higher than those in whole body. Levels of total n-6 fatty acids, however, were markedly lower in liver than in whole body in all treatment groups, suggesting that n-3 HUFAs were more effectively retained in liver than in muscle as reported by Torstensen et al. (2004) in Atlantic salmon. Consequently, the ratio of n-3/n-6 fatty acids was higher in liver than in whole body.

Red and white blood cell counts and hemoglobin were not influenced by added levels of fish oil. Hematocrit, however, was significantly lowest in fish fed the 9% added fish oil diet. In the same species, no effect of dietary fish oil levels up to 14% was observed on hematological parameters (Lim et al. 2006). Klinger et al. (1996), however, reported that catfish fed a menhaden oil diet had similar red blood cell count but significantly lower hematocrit than fish fed diets with soybean oil or beef tallow. In Nile tilapia, Yildirim-Aksoy et al. (2007b) observed that fish fed the fish oil diet had abnormally high red and white blood cell counts but similar hematocrit values compared with fish fed diets with other dietary lipid sources (linseed oil, corn oil, or beef tallow).

Erythrocytes from fish fed the diet with the highest (9%) level of added fish oil diet were significantly more resistant to hypotonic salt solutions than those from the groups fed lower levels of supplemental fish oil. Earlier studies in channel catfish (Klinger et al. 1996) and Atlantic salmon (Erdal et al. 1991) showed that elevated dietary levels of n-3 HUFAs resulted in increased resistance of erythrocytes to hypotonic salt solutions. The increase in cell wall strength may be because of increased flexibility and permeability of cell membranes as a result of incorporation of n-3 HUFAs. Increasing cell membrane flexibility has been shown to have immunostimulatory effect in fish (Balfry and Higgs 2001). Dietary fatty acids have also been shown to influence the fatty acid composition of erythrocyte membranes in rainbow trout (Kiron et al. 1994).

Fatty acids are well known to have multifunctional effects on metabolism and the immune system of fish (Balfry and Higgs 2001). The impacts that dietary fatty acids have on immune responses are complex and dependent on several factors. Dietary n-3 fatty acids affect immune functions in fish by influencing the physical properties of the cell membrane and the activity of membrane-associated receptors and enzymes. Any changes in viscosity and permeability of macrophage membranes can alter their cellular function (Obach et al. 1993). Lim et al. (2006) and Montero et al. (2003), however, found that dietary levels of fish oil

and lipid sources had no effect on lysozyme activity in channel catfish and gilthead seabream, respectively. In the present study, serum protein concentrations of fish fed 6 or 9% added fish oil diets were significantly higher than those of fish fed the control or 3% added fish oil diet. Enhanced lysozyme activity was obtained in fish fed diets supplemented with 3 or 6% fish oil, but significantly decreased in the group fed diet with 9% fish oil supplementation.

A significant increase in complement activity was obtained in catfish fed the diet with 3% supplemented fish oil. Increasing added fish oil levels to 6 or 9% adversely affected serum complement activity. In contrast, Lim et al. (2006) reported significant enhancement of alternative complement in catfish fed diets containing 14% fish oil. In gilthead seabream, the reduction of alternative complement activity was correlated with a dietary deficiency of n-3 HUFAs (Tort et al. 1996; Montero et al. 1998). The stimulatory effect of dietary n-3 HUFA has also been demonstrated in various fish species. Macrophages from head kidney of rainbow trout fed a diet enriched with n-3 fatty acids had greater migration-stimulating ability than those originating from trout fed a diet enriched with n-6 fatty acids (Ashton et al. 1994). In channel catfish, Lim et al. (2006) reported that 10% fish oil was sufficient to stimulate macrophage chemotaxis and phagocytosis. Increased intracellular killing of the bacterium *E. ictaluri* by head kidney macrophages has also been demonstrated in channel catfish fed diets containing 7% fish oil compared with those fed diets containing soybean oil or beef tallow (Sheldon and Blazer 1991). In contrast, our study showed that macrophage migration was depressed in the group fed 6% or more added fish oil diet compared with those of fish fed the control or 3% added fish oil diet. Immunosuppressive effects associated with excessive levels of n-3 HUFAs have also been reported in some fish species. Kiron et al. (1995) observed that excessive levels of n-3 HUFAs impaired the defense mechanism and increased the mortality of rainbow trout infected with *Aeromonas salmonicida*. Channel catfish fed a practical diet containing 2% menhaden fish oil were more susceptible to *E. ictaluri* than those fed diet lower in n-3 HUFAs (Li et al. 1994). Fracalossi and Lovell (1994) reported that at 28 C, catfish fed semipurified diets containing 7% menhaden oil (rich in n-3 HUFAs) or linseed oil (rich in 18:3n-3) were more susceptible to *E. ictaluri* challenge than those fed diets containing beef tallow, corn oil or an equal mixture of fish oil, corn oil and beef tallow. Erdal et al. (1991) reported that Atlantic salmon fed diets high in n-3 HUFAs had decreased antibody titers after vaccination and reduced survival after challenge with *Vibrio salmonicida*. In our study, fish fed a diet with 6% added fish oil had significantly lower mortality following *E. ictaluri* challenge than the groups fed diets with 3 or 9% added fish oil, but did not differ from that of the control. Based on n-3 HUFAs content (Table 1), our control diet appeared to contain some marine fish oil. The ratio of n-3 to n-6 fatty acids increased with increasing supplemental levels of fish oil; however, these values were considerably lower than those of fish oil-containing diets used in studies of Fracalosi and Lovell (1994) and Li et al. (1994). This may explain the differences obtained in this study and those of Fracalosi and Lovell (1994) and Li et al. (1994). Postchallenge antibody production against *E. ictaluri*, however, was not influenced by dietary fish oil levels, as reported by Lim et al. (2006) and Li et al. (1994).

Information on the effect of dietary lipids and their constituent fatty acids on immune responses and disease resistance in fish appeared to be inconsistent and sometimes contradictory. Several factors such as species, fish size, nutritional status, the composition of the experimental diets, nutrient interactions, feeding duration, and environmental factors may account for the discrepancies among research results. Thus, further studies to reveal how dietary fatty acids affect immune system function and disease resistance at different life stage of various fish species are warranted.

In the present study, growth performance (weight gain, feed utilization, and survival) of channel catfish was not influenced by supplementation of fish oil at levels of 0, 3, 6, and 9% to a commercial catfish diet. Hematocrit was lowered in fish fed a diet with 9% added fish

oil. The resistance of erythrocytes to hemolysis in hypotonic solutions increased with increasing dietary levels of fish oil and erythrocytes from fish fed 9% added fish oil diet were the most resistance. This level of supplemental fish oil, however, resulted in lower hematocrit. Immunostimulatory effects of dietary fish oil (n-3 HUFA) supplementation appeared to vary from 3 to 6%. Immunosuppressive effects associated with excessive levels of fish oil were also observed at levels from 6 to 9%, depending on the immune parameters evaluated. Resistance of fish to *E. ictaluri* challenge was not influenced by additional levels of fish oil. There is a need for more basic understanding of the lipid metabolism of catfish and the mechanisms by which dietary levels of n-3 HUFAs influence immune cells such as macrophages and lymphocytes, and specific immune functions, and disease resistance.

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